

DNA GLOBALFILER INTERPRETATION GUIDELINES

A. SCOPE

- A.1 The interpretation of results in casework is necessarily a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. However, it is important that the laboratory develops and adheres to minimum criteria for interpretation of analytical results. These criteria are based on validation studies, literature references, and casework. It is to be expected that these interpretation guidelines will continue to evolve as the technology and collective experience of the laboratory grows.
- A.2 The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that:
- A.2.1 Conclusions in casework reports are scientifically supported by the analytical data, including that obtained from appropriate controls.
- A.2.2 Interpretations are made as objectively as possible, consistently from analyst to analyst, and within established limits.
- A.3 The goal of the evaluation and interpretation of amplified STR data is to determine the DNA profile(s) of the donor(s) of the questioned samples for comparison to reference sample profile(s).
- A.3.1 A peak is defined as a distinct, triangular section of an electropherogram.
- A.3.2 Genotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus

B. PRELIMINARY EVALUATION OF GLOBALFILER DATA

B.1 ANALYTICAL THRESHOLD

3130: 75 RFU
3500xL: 100 RFU

B.2 STOCHASTIC THRESHOLD

3130-1: 200 RFU / 3 sec injection
260 RFU / 5 sec injection
370 RFU / 8 sec injection

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3130-2: 215 RFU / 3 sec injection
345 RFU / 5 sec injection
530 RFU / 8 sec injection

3500xL: 555 RFU / 24 sec injection

B.3 **LADDER**

The ladder used for sizing must be evaluated to determine that all peaks type correctly, no spikes exist in the sizing range, and peak heights are at or above the analytical threshold. This evaluation may be performed by GeneMapper ID-X.

B.4 **INTERNAL SIZE STANDARD**

The internal size standard utilized is GeneScan-600 LIZ. The GeneScan-600 LIZ must be evaluated to determine that the expected pattern of peaks is present; this evaluation may be performed by GeneMapper ID-X.

B.5 **INTERPRETATION OF CONTROL SAMPLES**

B.5.1 **REAGENT CONTROLS**

A reagent control tests for the possible presence of contamination of the extraction reagents and/or supplies by an adventitious source of DNA. The adventitious DNA can be non-amplified DNA or PCR product. If only a single peak (not attributable to a spike or other artifact) exists in the reagent control electropherogram between 60 and 460 bp and there are no signs of any other peaks, this will not be reported as contamination unless this sample has a quantitation value of 10^{-3} ng/ μ L or higher. Conversely, if there is a single peak in the reagent control electropherogram (not attributable to a spike or other artifact) but signs of other peak(s) are visible, this will be reported as contamination, even if this reagent control did not have a quantitation value. However, due to the low level of DNA present and the inability to draw any conclusions for this single result, this contamination will typically not affect any conclusions drawn for corresponding questioned samples. If, between 60 and 460 bp, a reagent blank exhibits multiple peaks at or above the analytical threshold, not attributable to spikes or other artifacts, the questioned samples extracted with the reagents contained in the reagent blank will be considered inconclusive for match purposes unless it can be determined that the contamination is isolated to the reagent control and did not affect the evidentiary samples. This can be accomplished through additional samples extracted simultaneously that exhibit no peaks, e.g. a second reagent control, water control, or a sample containing no

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DNA. Where possible, the analyst may go back to the earliest item of evidence associated with the blank e.g. raw evidence material, to try to reproduce or eliminate the contamination. Efforts should be made to determine the source of contamination. If contamination cannot be deemed isolated to just the reagent blank and re-analysis is not possible then these questioned samples cannot be used for inclusions or exclusions. Reagent blanks containing spikes between 60 and 460 bp may be re-injected. Spikes or other artifacts that fall outside these areas are not significant. The Technical Leader must be informed of any contamination event and the contamination log must be filled out. Contamination events will be documented in the case package and report.

B.5.2 NEGATIVE CONTROL

A negative control (amplification blank) tests for the possible presence of contamination occurring during amplification set-up. If, between 60 and 460 bp (GlobalFiler), a negative control exhibits any peaks at or above the analytical threshold not attributable to a spike or other artifact, the DNA specimens amplified at the same time as the negative control will be considered inconclusive for match purposes unless it can be determined that the contamination is isolated to the negative control and did not affect the evidentiary samples. This can be accomplished through additional samples extracted simultaneously that exhibit no peaks, e.g. a reagent control, water control, or a sample containing no DNA. Where possible, the analyst may go back and re-amplify all samples associated with this amplification negative control. If contamination cannot be deemed isolated to just the amplification blank and re-analysis is not possible then these samples cannot be used for inclusions or exclusions. Negative controls containing spikes or other artifacts between 60 and 460 bp may be re-injected. Spikes or other artifacts that fall outside these areas are not significant. The Technical Leader must be informed of the contamination event. Contamination of the negative control will be documented in the case package.

B.5.3 POSITIVE CONTROL

DNA Control 007 is a positive control for the GlobalFiler STR amplification kit; this control can be used to evaluate the performance of the amplification and typing procedure.

D3S1358	VWA	D16S539	CSF1PO	TPOX	INDEL	AMEL	D8S1179
15,16	14,16	9,10	11,12	8	2	X,Y	12,13

D21S11	D18S51	DYS391	D2S441	D19S433	THO1	FGA	D22S1045
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28,31	12,15	11	14,15	14,15	7,9.3	24,26	11,16
D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338
11	11	7,12	17,25.2	12,15	13,16	18,19	20,23

Table 1. DNA profile for 007 positive control.

If the positive control does not exhibit the STR typing results listed in Table 1, the following steps will be taken:

- B.5.3.1 If there appears to be an injection or electrophoretic problem, re-inject the positive control. Alternatively, the amplified product may be re-set up for electrophoresis.
- B.5.3.2 If re-injection of the positive control does not resolve the problem and there may be amplification issues, all samples co-amplified with this control will be considered inconclusive for matching purposes at each locus where the positive control did not exhibit results at or above the analytical threshold, unless a QC sample (an in-house sample with a known profile) was amplified concurrently and that sample gave the expected profile with all results above at or above the analytical threshold. If sufficient DNA remains from samples co-amplified with a failed control and no QC sample was co-amplified, then re-amplification is appropriate. If the positive control yields accurate typing results when re-amplified, then the samples re-amplified with this control will be considered acceptable for matching purposes.
- B.5.3.3 If results of the positive control show an incorrect allele call, all samples co-amplified with this control will be considered inconclusive.
- B.5.3.4 In some instances, it may be necessary to target multiple concentrations of the positive control for amplification in order to obtain a full DNA profile. The amplification is deemed acceptable as long as one of the positive controls produces a profile with all results at or above the analytical threshold.

B.6 PEAK HEIGHT RATIOS

Peak height ratios of heterozygote alleles are defined as the ratio of the lower peak's height to the higher peak's height, typically expressed as a percentage.

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3130 CE: When both alleles have peak heights greater than 500 RFU, then it is expected that the peak height ratio will be equal to or greater than 65%, otherwise a mixture may be indicated. When at least one allele from a heterozygote pair has a peak height less than 500 RFU, a peak height ratio of 40% may be observed and is acceptable.

3500xL CE: The minimum Peak Height Ratio (minPHR) that will be allowed for low input amounts of DNA is 18% (although, the consideration of any PHR below 33% must be taken to the DNA Technical Leader as the minPHR for 0.125 ng of template DNA at an AT of 100 RFU is 33%).

The desired target for DNA input is between 0.5 ng – 1.0 ng with a minPHR of 45%. (PHR's are at least 70% for single source samples with an input of 1 ng DNA).

Average heterozygous peak heights at 0.5 ng – 1.0 ng of input DNA is between 2758 – 4832 RFU's.

Homozygote allele peak heights are approximately twice that of heterozygotes as a result of a doubling of the signal from two alleles of the same size.

B.7 DESIGNATION

B.7.1 LOCUS AND ALLELE DESIGNATION

The GeneMapper ID-X program will be used for the locus and allele designations of the raw data. The examiner must review the GeneMapper ID-X calls (except ladders) and create a table of results from this review.

B.7.2 OFF LADDER VARIANTS

Off ladder alleles that fall between alleles within the ladder will be designated in accordance with guidelines of the International Society for Forensic Haemogenetics. Off ladder (OL) calls are first converted to size in base pairs (bp), then compared to the size of the appropriate ladder alleles and the allelic designation determined. If the OL is not a "perfect" repeat, but rather varies by 1, 2 or 3 bp from a ladder allele, then it will be designated as an integer of that variation. For example, if a green OL peak size is 238.39 bp, and the 36 allele of the **D21S11** ladder is 236.32 bp, then the peak will be designated a **D21S11 36.2**. If an allele falls above the largest or below the smallest peak of the sizing ladder, the allele will be designated as either greater than (>) or less than (<) the respective ladder allele.

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Non-artifactual off-ladder alleles will generally conform to the same overall guidelines described above. An analyst must re-inject (or re-amplify) any off ladder alleles in a sample for verification unless the results from that sample will not be interpreted.

B.7.3 ARTIFACTS

Artifacts can occur and need to be recognized. These may include, but are not limited to, the following: stutter, non-template nucleotide addition, pull-up and spikes.

B.7.3.1 STUTTER

In addition to an allele's primary peak, artifactual minor "stutter" peaks can occur at four-base intervals. The most common stutter peaks observed in all loci are four bases smaller than the primary peak ("n-4"). It is also possible to see additional "n+4" peaks (four bases larger), especially when excessive amounts of DNA are amplified. In addition, samples amplified using GlobalFiler may exhibit n-3 and n+3 at D22S1045 (a trinucleotide repeat) and n-2 (in addition to n-4) at both SE33 and D1S1656.

Stutter peaks are evaluated by examining the ratio of the stutter peak height to the height of the appropriate adjacent allele, expressed as a percentage. The height of stutter peaks can vary by locus, and longer alleles within a locus generally have a higher percentage of stutter. When interpreting GlobalFiler data, locus specific stutter percentages will be utilized (Table 2).

Table 2. Locus specific minus and plus stutter percentages for GlobalFiler (manufacturer's published stutter filters)

Locus	Minus stutter filter %	Locus	Plus stutter filter %
D3S1358	10.98	D3S1358	
vWA	10.73	vWA	
D16S539	9.48	D16S539	
CSF1PO	8.77	CSF1PO	
TPOX	5.55	TPOX	
D8S1179	9.60	D8S1179	
D21S11	10.45	D21S11	
D18S51	12.42	D18S51	

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DYS391	7.43	DYS391	
D2S441	8.10	D2S441	
D19S433	9.97	D19S433	
TH01	4.45	TH01	
FGA	11.55	FGA	
D22S1045 (-3)	16.26	D22S1045 (+3)	6.69
D5S818	9.16	D5S818	
D13S317	9.19	D13S317	
D7S820	8.32	D7S820	
SE33 (-4)	14.49	SE33	
SE33 (-2)	3.97	D10S1248	
D10S1248	11.46	D1S1656	
D1S1656 (-4)	12.21	D12S391	
D1S1656 (-2)	2.45	D2S1338	
D12S391	13.66		
D2S1338	11.73		

While the internal validation of GlobalFiler showed plus stutter at just a few loci. The manufacturer's developmental validation demonstrated that plus stutter can occur in all GlobalFiler loci except D7S820, TH01 and TPOX; however, this stutter was typically present only at very low frequencies. Analysts can use the manufacturer's plus stutter information to assist them in their interpretation of GlobalFiler results (Table 3).

Table 3. Locus specific plus stutter percentages observed in the manufacturer's population study with the GlobalFiler Kit (population database size: 1194 samples)

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Loci	Observations	Mean	Standard deviation	Minimum	Maximum†
CSF1PO	19	1.533	0.496	0.85	2.64
D10S1248	40	2.076	1.104	0.56	6.77
D12S391	15	2.582	1.162	0.73	5.21
D13S317	28	1.803	1.232	0.57	7.06
D16S539	12	1.77	1.143	0.64	3.75
D18S51	27	3.106	2.25	0.62	9.8
D19S433	8	2.574	1.181	1.11	4.85
D1S1656	60	1.583	1.072	0.62	8.07
D21S11	28	1.655	1.064	0.64	4.38
D22S1045 (N + 3)	1002	4.258	0.8103	0.85	7.34
D2S1338	14	3.516	2.06	1.13	8.34
D2S441	24	2.313	3.126	0.54	15.31
D3S1358	8	1.798	1.137	0.56	4.07
D5S818	48	1.493	0.817	0.73	5.88
D8S1179	46	1.6	0.778	0.6	4.36
DYS391	10	2.556	1.69	0.95	6.32
FGA	24	2.569	2.265	0.81	11.37
SE33 (N + 4)	76	2.48	1.164	0.85	6.13
WWA	8	1.495	1.426	0.65	4.97

† The maximum value represents outliers. The vast majority of the data (95%) obtained was within the mean ± 2 standard deviations.

Analyzed peak heights above the optimal range (approximately 75-6000 RFU for 3130 data, and 100-8000 RFU for 3500xL data) may be “off-scale” in the raw data, meaning that the CCD camera may have been saturated. While the GeneMapper ID-X software will alert the analyst to any off-scale raw data peaks, the analyzed peak may be assigned a lower value due to smoothing and base-lining functions. Therefore, the observed percent stutter will be inaccurately high. If the stutter peak is greater than the maximum predicted and the primary peak is above the saturation threshold of the CCD camera, and/or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be re-amplified with less input DNA or re-injected for a shorter period of time.

Stutter peaks overlapping an area of elevated baseline may exhibit increased percentages. Elevated baselines may indicate an improperly functioning spectral.

B.7.3.2 **NON-TEMPLATE NUCLEOTIDE ADDITION (–A)**

Amplification conditions have been set to maximize the non-template addition of a 3' terminal nucleotide by Taq DNA polymerase. Failure to attain complete

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terminal nucleotide addition results in “peak splitting” resulting in two peaks one base apart. This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions. Excepting micro-variants, the presence of peaks differing by one base pair is diagnostic of this problem.

B.7.3.3 ARTIFACTS INTRINSIC TO THE GLOBALFILER KIT

Reproducible artifacts intrinsic to the GlobalFiler Kit have been identified at the TH01, TPOX, D5S818 and D3S1358 loci. These artifacts are DNA target dependent, i.e. they occur only in the presence of DNA and not in negative controls. Although these artifact peaks are typically present at a low level, they may be observed depending on DNA input amount.

TH01: The artifacts are observed at n-12 nucleotides from the true TH01 peaks and are likely caused by secondary structure. There is a direct correlation between the signal intensities of the n-12 artifacts and the TH01 alleles with artifact peak heights that range from 0.4% to 0.9% of the TH01 allele peak heights.

TPOX: The artifacts are observed at n-24 nucleotides from the true TPOX peaks and are likely caused by secondary structure.

D5S818: The artifacts are approximately 180 bp and likely due to a non-STR amplification by-product.

D3S1358: The artifacts are approximately 132 bp and likely due to a non-STR amplification by-product.

B.7.3.4 OTHER ANOMALIES

In addition to amplification artifacts described above the following anomalies (non-reproducible, intermittent occurrences) can arise during electrophoresis and analysis:

Significant room temperature fluctuation may result in electrophoretic variations between injections such that allelic ladder peaks differ by more than 0.5 bp from allelic peaks in other injections. This will disrupt sample analysis using the GeneMapper ID-X software. Analyzing samples with an injection of allelic ladder nearest the questioned samples may alleviate this problem. If desired, the sample(s) and an allelic ladder may be re-injected to confirm the typing.

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Peaks of the same size and similar height (within an order of magnitude of each other) in multiple dye channels are not the result of dye-labeled DNA and do not represent a spectral problem. These are artifacts typically due to the presence of fluorescent material in either the formamide or the POP-4 polymer. These peaks can be shown to be artifacts by re-injection of the sample.

Small artifactual peaks can appear in other colors under true peaks. This phenomenon is termed "pull-up". Pull-up is a result of spectral overlap between the dyes, which is normally corrected for by the spectral. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at the same size as the true peak. Pull-up can occur as a result of the amplification of excess input DNA resulting in off-scale peaks (see above). The spectral may not fully compensate with off-scale data.

B.8 INTERPRETATION OF RESULTS

B.8.1 DETECTION AND INTERPRETATION OF SINGLE SOURCE SAMPLES

A sample is consistent with being from a single source if each locus typed has only one or two alleles. For an apparent single source questioned sample, compare the results obtained from the questioned sample with the results from the known sample(s) in the case. The determination of inclusions or exclusions is the responsibility of the analyst working the case. This determination is based on all tests and observations made for that sample. In general, if any STR locus doesn't match between a single source questioned sample and a reference sample, then an exclusion can be made regardless of how many other loci match. An exception to this can occur with partial DNA profiles due to DNA degradation, inhibition of amplification, and/or low template quantity and in paternity testing because of the possibility of mutational events.

Statistical interpretation may be applied at loci when both alleles of a heterozygote pair are at or above the analytical threshold. If a single peak at a locus is observed and it is below the appropriate stochastic threshold, then it is possible that if the locus is heterozygous, the sister allele may not have been detected. In this case the 2p statistical approach will be used to provide the weight of a match at that particular locus.

B.8.2 DETECTION OF MIXTURES

Samples may contain DNA from more than one individual. A sample is consistent with being a mixture if it exhibits one or more of the following characteristics at more than one locus:

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- B.8.2.1 More than two alleles are present at a locus after stutter, tri-alleles, and other artifact and anomaly considerations have been evaluated and dismissed as possible causes. If it is not clear whether these allele(s) are from known artifacts/anomalies or a second individual, then the analyst may instead report that the results indicate "at least one contributor."
- B.8.2.2 A peak is present at a stutter location and its height is greater than the locus specific stutter percent determined for GlobalFiler.
- B.8.2.3 Severely imbalanced peak heights exist for sister alleles of heterozygous genotypes within the profile. See section **B.6** of this document for further guidance.

B.8.3 INTERPRETATION OF MIXED SAMPLES

An individual's contribution to a mixed biological sample is generally proportional to their quantitative representation within the DNA typing results. Accordingly, depending on the relative contribution of the various contributors to a mixture, the DNA typing results may potentially be further refined. The interpretation applied to a mixed sample by the analyst in each particular case should be based upon all relevant information.

- B.8.3.1 Inconclusive / uninterpretable data at individual loci or an entire multi-locus profile must not be used in statistical analysis.
- B.8.3.2 The determination of which alleles/loci are suitable for comparison and use in statistical analysis must be made prior to any comparison to the known reference profile(s), other than those of assumed contributors when analyzing intimate samples.
- B.8.3.3 If the evidence originates from an item, or stain where a reasonable expectation of the presence of an individuals' DNA exists such as an intimate sample i.e. body swab, tampon, or catheter; or blood stained clothes from an assaulted victim, a profile foreign to the source of that sample/stain may be readily inferred. This can be accomplished by subtracting the DNA contribution of the assumed contributor from the mixed profile resulting in a deduced foreign DNA profile. Peak heights, peak height ratios, and the stochastic threshold need to be considered when deducing a profile.
- B.8.3.4 For a distinguishable mixture i.e. a mixture that has a distinct contrast in signal intensities (peak heights) among the different

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contributors' alleles, a dominant and/or minor contributor can be determined based on peak heights as well as peak height ratios. Once determined, these profiles can be compared to known reference profile(s) for inclusionary and exclusionary purposes as would a single source profile.

- B.8.3.5 For a distinguishable mixture, a dominant contributor(s) profile may be suitable for statistical analysis even in the presence of inconclusive minor contributor results.
- B.8.3.6 In general, heterozygous alleles attributed to either a dominant or minor contributor should meet the single source peak height ratio expectations as described in section **B.6**. The analyst should keep in mind that the peak height ratio expectations of a minor contributor may be reduced due to stochastic peak height variation and the additive effects of peak sharing (e.g. minor peak and stutter peaks).
- B.8.3.7 For an indistinguishable mixture, i.e. a mixture that does not have a distinct contrast in signal intensities among different contributors, one may be able to determine whether an individual can be excluded as a source of DNA in the mixture or can be included as a possible source of DNA in the mixture. If the known DNA profile contains an allele or alleles not present in the mixture profile, then an exclusion may be made provided there is no reason to believe allelic dropout has occurred. If an inclusion is made, a mixture statistic (specifically a combined probability of inclusion (CPI)) accounting for all of the possible combinations of detected alleles at each locus should be applied. The number of unrelated individuals who could have contributed to the mixture will then be reported. However, if there is an indication that allelic dropout has occurred or may have occurred, this locus will not be used in statistics; see guidelines B.8.3.8 and B.8.3.9 for further explanation of this.

Mixed dominant DNA profiles consisting of two or more individuals may also be interpreted. For example, a mixed dominant DNA profile consisting of two individuals at approximately equal contributions may be determined in a mixture consisting of three or more individuals if there is a distinct difference between the contribution of the two individuals present in the mixed dominant profile and the individual(s) providing the minor or trace DNA results. If an inclusion is made for the mixed dominant profile, a CPI

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statistic accounting for all of the possible combinations of detected alleles at each locus should be calculated.

When a differential extraction is performed on a non-intimate sample and that sample is divided into two fractions, a sperm fraction and an epithelial fraction, the results from one of those fractions may be used to assist in the interpretation of the other fraction. For example, if a dominant male DNA profile is obtained from the sperm fraction of a clothing stain but the results from the epithelial fraction of that same stain do not have an interpretable female DNA profile (due to carryover from the sperm fraction), the dominant DNA profile from the sperm fraction can be subtracted from the epithelial fraction. By subtracting this profile from the mixed DNA results, an interpretable female DNA profile may be deduced in the epithelial fraction. Peak heights, peak height ratios, and the stochastic threshold need to be considered when deducing a profile in this manner.

B.8.3.8 DNA results indicating a mixture of two individuals displaying two or three peaks all above the stochastic threshold, may or may not have all results accounted for within these peaks. Because allele stacking is a possibility, having all of the alleles above the stochastic threshold does not mean that allelic dropout did not occur with one or more of the contributors to the mixture. However, if by looking at the peak heights at this locus and the overall DNA profile, one can opine that no alleles are missing, then this locus may be used in the CPI statistical calculation. If multiple possible combinations exist for a dominant or minor contributor in this mixture, then this locus must still be left out of the single source statistic (random match probability (RMP)).

B.8.3.9 When using CPI to calculate the probability that a randomly selected person would be included as a contributor to the mixture, loci with alleles below the stochastic threshold may not be used for statistical purposes. In these instances, the potential for allelic dropout raises the possibility of contributors having genotypes not encompassed by the interpreted alleles. For example, if the DNA results indicate a mixture of two individuals and a locus has four alleles above the analytical threshold present, but one or more of these alleles is below the stochastic threshold, this locus cannot be used in the CPI statistic. However, if a dominant contributor can be determined as having both alleles above the analytical threshold,

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then a RMP statistic may be applied to the dominant contributor's profile at this locus.

- B.8.3.10 The overall profile should be considered when interpreting a mixed profile, e.g. peak heights and ratio range for minor to dominant alleles.
- B.8.3.11 For mixtures in which minor contributors are determined to be present, a peak in a stutter position may be determined to be 1) a stutter peak, 2) an allelic peak or 3) indistinguishable as being either an allelic or stutter peak. This determination is based principally on the height of the peak in the stutter position and its relationship to the stutter percentage expectations.

Generally, when the height of a peak in the stutter position significantly exceeds the stutter percentage expectation for a given locus, that peak is consistent with being of allelic origin and should be designated as an allele. However, this decision should be based on viewing the DNA profile in its entirety and taking the possibility of elevated stutter due to a combination of plus and minus stutter into consideration.

If a peak is at or just above the stutter filter, depending on whether there is an indication of a minor profile with similar RFUs, it may be designated as a stutter peak and filtered accordingly, or designated as indistinguishable as stutter or a true allele.

B.8.4 PARTIAL SINGLE SOURCE STR PROFILES

The possibility exists that not every locus will amplify. This can occur if the DNA is of limited quantity, severely degraded or if the DNA sample contains PCR inhibitors. Since loci are independent, any locus that shows results can be evaluated. Statistical interpretation may be applied at loci when both alleles of a heterozygote pair are at or above the analytical threshold. With lower levels of DNA, peak imbalance and allelic dropout are more likely. If a single peak at a locus is observed that is below the stochastic threshold, then it is possible that if the locus is heterozygous, the sister allele may not have been detected. This locus can also be evaluated for both exclusionary and inclusionary purposes with the "2p" statistical method being applied if an association with a known sample is subsequently determined. If there are insufficient results in the partial STR profile to provide any statistical interpretation for inclusionary purposes, then the results should be reported as "due to a low level of DNA, no conclusions can be offered".

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B.8.5 PARTIAL MIXED STR PROFILES

It is possible in a mixture that the entire profile from one of the contributors is not represented at all loci. This may be due to allelic dropout or allele masking in a stutter position. In this situation an individual cannot necessarily be excluded from contributing to the mixed DNA profile and should be reported as "cannot be excluded as a source of the mixed DNA profile obtained from ..." The affected areas will be left out of the statistical calculation. If there are insufficient results in the partial mixed STR profile to provide any statistical interpretation for inclusionary purposes, then the results should be reported as "due to a low level of DNA, no conclusions can be offered".

B.9 CONCLUSIONS

B.9.1 Single source inclusions: the questioned sample yields a single source DNA profile and the known sample has the same profile. The following applies to a single source dominant component of a mixture, a single source minor component of a mixture and a single source foreign deduced profile also:

B.9.1.1 If the frequency of the matching profile is greater than 1 in 8 trillion (generated by applying a 99.9% confidence interval to the world population) in each of the four populations examined using the NIST database (Caucasian, Hispanic, African American and Asian) then the estimated frequency of this matching DNA profile will be reported and a source attribution statement regarding this individual will be made.

B.9.1.2 If the frequency of the matching profile is less than 1 in 8 trillion in one or more of the four populations examined using the NIST database (Caucasian, Hispanic, African American and Asian) then the source of the known sample will be reported as "cannot be excluded" and the estimated frequency of this matching DNA profile will be provided.

B.9.2 Single source exclusions: the questioned sample and the known sample do not contain the same DNA profile. The source of the known will be excluded as being the source of the questioned sample when the obtained profiles differ in at least one locus. (Note: exceptions include low levels of DNA/partial profiles).

B.9.3 Mixture inclusions and exclusions: the questioned sample yields an interpretable DNA mixture or a mixed dominant DNA profile that contains the DNA profile of the known sample. The source of the known sample will be reported as "cannot be excluded" from contributing to the mixture or mixed

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dominant DNA profile and the estimated number of individuals who could have contributed to this mixture will be provided. Conversely, if the mixture does not contain the DNA profile of the known sample, then this individual will be excluded as being a source of the mixture. (Note: exceptions include low levels of mixed DNA/mixed partial DNA profiles).

- B.9.4 **Mixture notations:** when interpreting data from mixture DNA results, clear notes must be made as to the assumptions and reasoning behind the interpretation(s) and conclusion(s) reached. Ordinarily these will be handwritten on the associated electropherogram.
- B.9.5 **Low-level:** when an overall low-level profile is obtained, results may be deemed inconclusive or uninterpretable when multiple loci are missing one or both alleles, the alleles present are below the stochastic threshold, an analyst cannot conclusively determine that only one individual is contributing alleles to the observed results, etc.
- B.9.6 **No results:** a sample may be reported out as having no results obtained when no signal at or above the analytical threshold is detected.

C. REFERENCES

- C.1 DNA Technology in Forensic Sciences, National Research Council, National Academy Press, 1992
- C.2 The Evaluation of Forensic DNA Evidence, National Research Council, National Academy Press, 1996.
- C.3 Forensic DNA Typing, John M. Butler, Academic Press 2001 and 2005.
- C.4 SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories, Approved 1/14/10.
- C.5 GlobalFiler PCR Amplification Kit User Guide, 2014, Applied Biosystems by Life Technologies; Developmental Validation included on pages 54-135.
- C.6 WCSO GlobalFiler Validation Binders, 2015-2016, volumes 1-5.
- C.7 [WCSO GlobalFiler Validation Studies, 2018.](#)

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